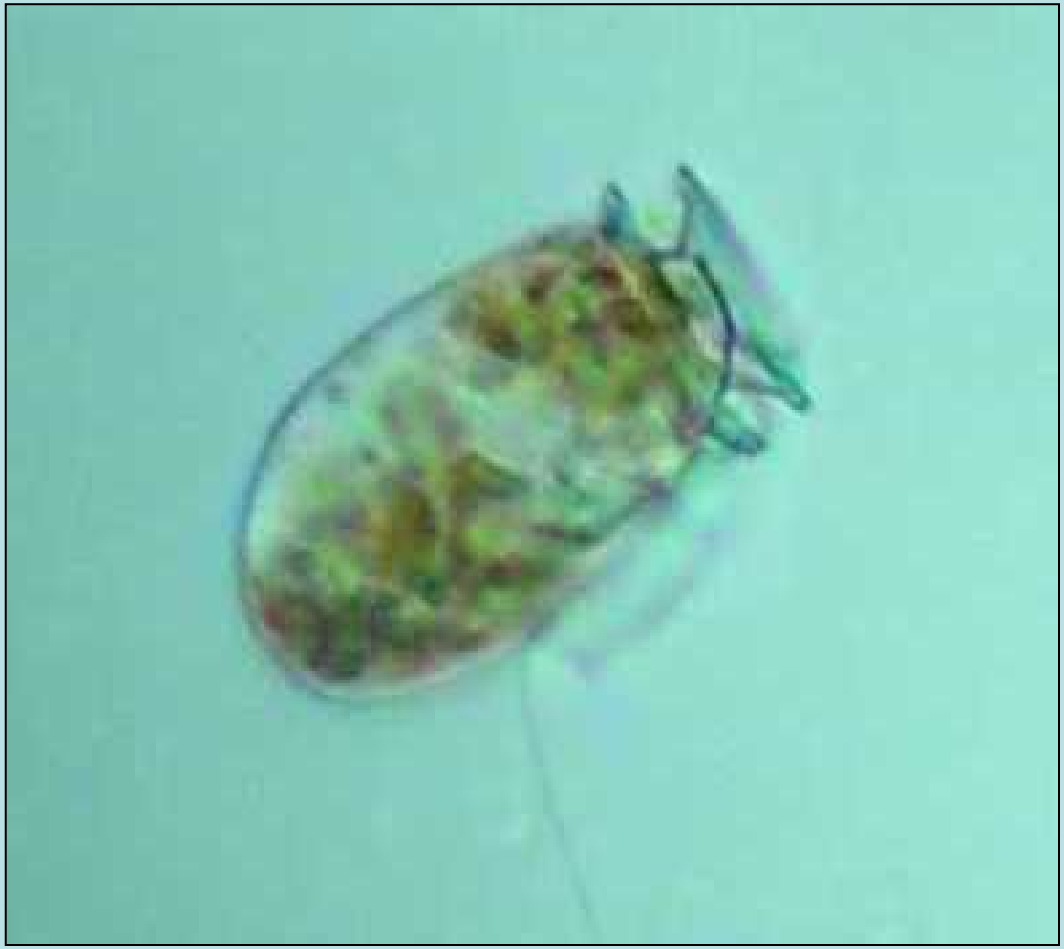


Dinophysis acuminata in Delaware's Inland Bays and coastal waters 2001-2015

Ed Whereat, Program Coordinator,
University of Delaware Citizen Monitoring Program
Delaware Sea Grant Marine Advisory Service

The UD Citizen Monitoring Program is a volunteer-based water quality monitoring program started in 1991. We added Harmful Algae Bloom (HAB) monitoring in 2001 in order to:

1) Increase the spatial and temporal coverage of monitoring for Harmful Algae Blooms (HABs) in the Inland Bays.

2) Improve the predictive capability of HAB occurrence for water quality management and public health alerts.

3) Improve public understanding of phytoplankton blooms by engaging citizen volunteers as Inland Bays stewards.

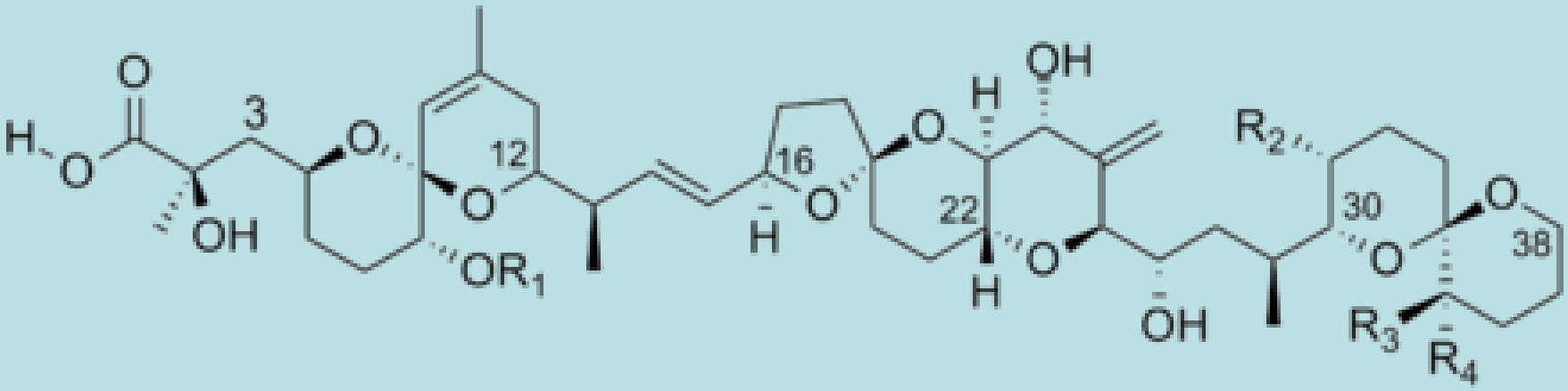
4) Provide significant samples to the DNREC and/or researchers at UD CEOE and other institutions.

Dinophysis acuminata, a dinoflagellate, is an emerging threat to the safety of shellfish consumption in the US.

Diarrhetic Shellfish Poisoning (DSP) is caused by Okadaic acid (OA) and it's analogues from toxin producing *Dinophysis*. The toxins are lipophilic and accumulate in fatty tissues of shellfish. These compounds are potent phosphatase inhibitors that rapidly cause inflammation of the intestinal tract and diarrhea in humans. They are also tumor promotors in animal test systems. The toxins are heat stable and remains in cooked products.

The links between DSP, toxins and *Dinophysis* have been a subject of research since the 1960's following outbreaks of illness related to the consumption of mussels in Europe and Asia. A variety of toxins were elucidated in the 1990's. Methods for detection have improved in the last several years.

Okadaic Acid and Dinophysis toxins



OA:	R_1=H	R_2=CH_3	R_3=H	R_4=H
DTX-1:	R_1=H	R_2=CH_3	R_3=CH_3	R_4=H
DTX-2:	R_1=H	R_2=H	R_3=H	R_4=CH_3
DTX-3:	R_1=Acyl	R_2=CH_3	R_3=CH_3	R_4=H

(Note that other toxins that do not cause diarrhea may also be present: Pectenotoxins)

DSP is under-diagnosed since symptoms are similar to gastroenteritis caused by food-borne pathogenic bacteria or viruses.

Dinophysis is mixotrophic. It can do photosynthesis, but largely relies on eating ciliates, which feed on smaller flagellated phytoplankton. The ability to culture this “food chain” and thus, to facilitate research, is recent. Growth and toxin production in *Dinophysis* seems to be stimulated by elevated levels of ammonium and phosphate.

The toxins may help immobilize the prey of *Dinophysis* and/or serve as a feeding deterrent for zooplankton that prey on *Dinophysis*.

Toxin production and profiles, cell morphology and genetic similarity vary among strains from different geographic locations, so the issues are quite complicated. For the time being, it’s best to call it the *Dinophysis acuminata* complex.

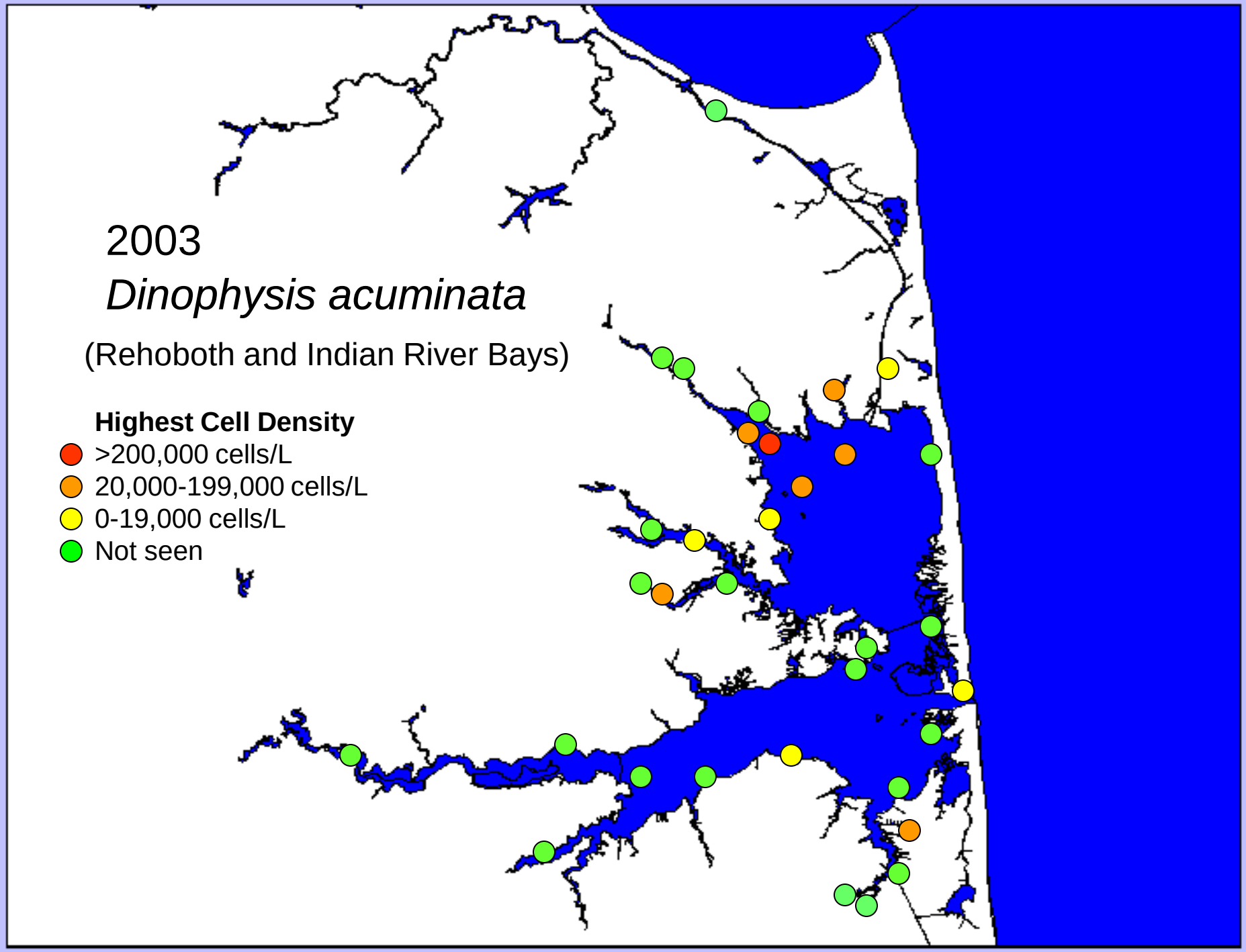
In the US, the first occurrences of shellfish harvesting closures due to OA concentrations exceeding FDA regulatory guidelines occurred on the Texas Gulf coast in 2008, the Puget Sound (WA) and Long Island Sound (NY) in 2011, and on Cape Cod (MA) in 2015. Confirmed cases of DSP have occurred in the Puget Sound (WA).

We appear to have relatively weak toxin producers in the mid-Atlantic states, but we see cell densities far higher than thresholds of concern in areas that DSP is a problem.

Overall, it appears that the risk of shellfish toxicity in the mid-Atlantic states is low, but the situation warrants continued surveillance, especially in the face of a growing shellfish aquaculture industry in VA, MD, DE and NJ.

An informal Mid-Altantic working group, with representatives from DE, MD, VA and US FDA has formed over the past few years. Researchers capable of addressing these factors have been taking a closer look at local *Dinophysis* populations. The first cultures established from the area were from DE this summer.

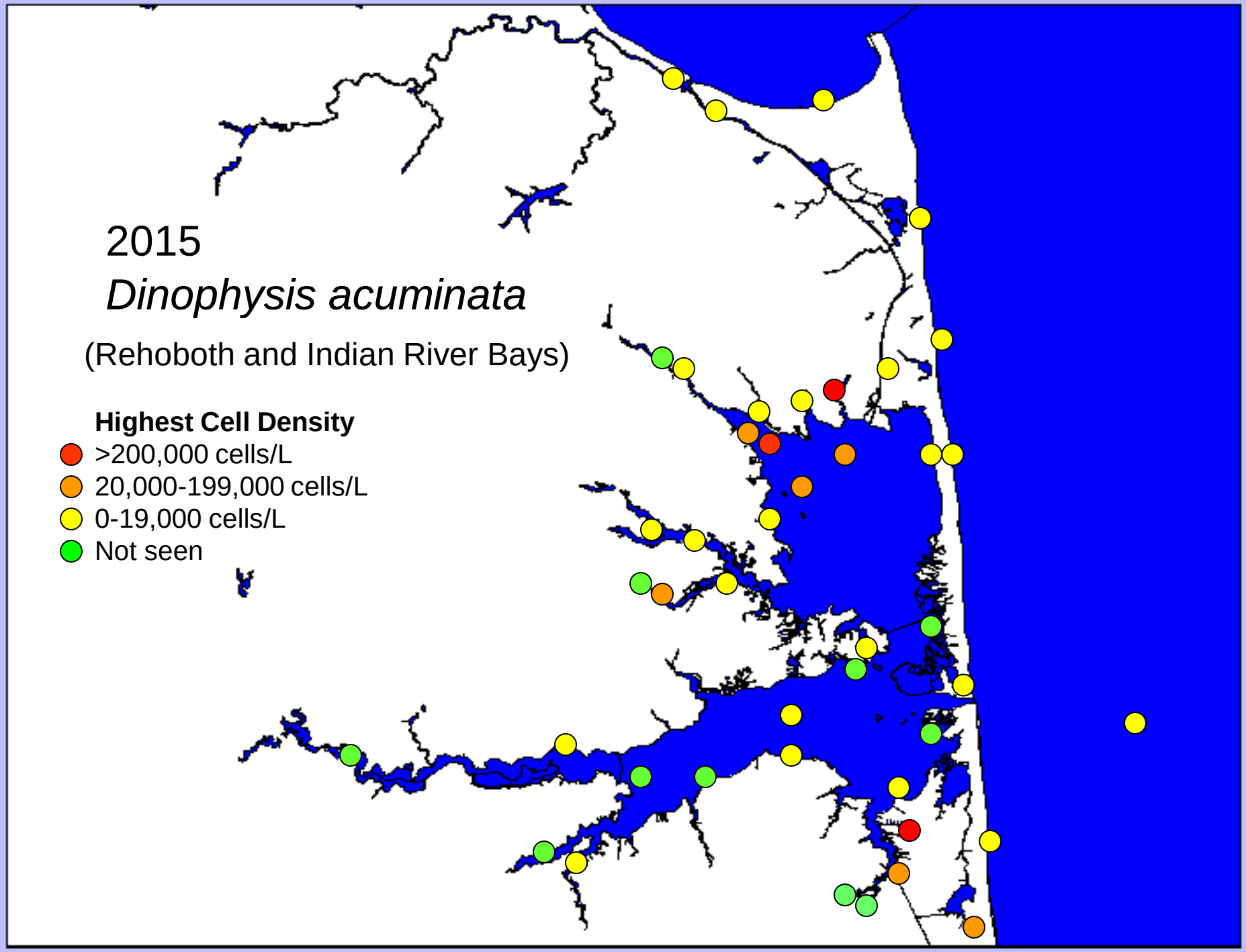
Jonathan Deeds (US FDA) – Toxin analysis, FDA shellfish regs.
Sara Handy (US FDA) – Genetic relatedness of *Dinophysis* species
Jennifer Wolny (MD DNR) – Microscopic ID and Morphometrics
Juliette Smith (VIMS) – Culturing and Toxin analysis
Todd Egerton (ODU) – Microscopic ID and Morphometrics
Joe Pitula and Detbra Rosales (UMES) – Genetic detection in MD
Ed Whereat (UD) – Microscopic ID
Mike Bott (DNREC) – Shellfish and water sampling

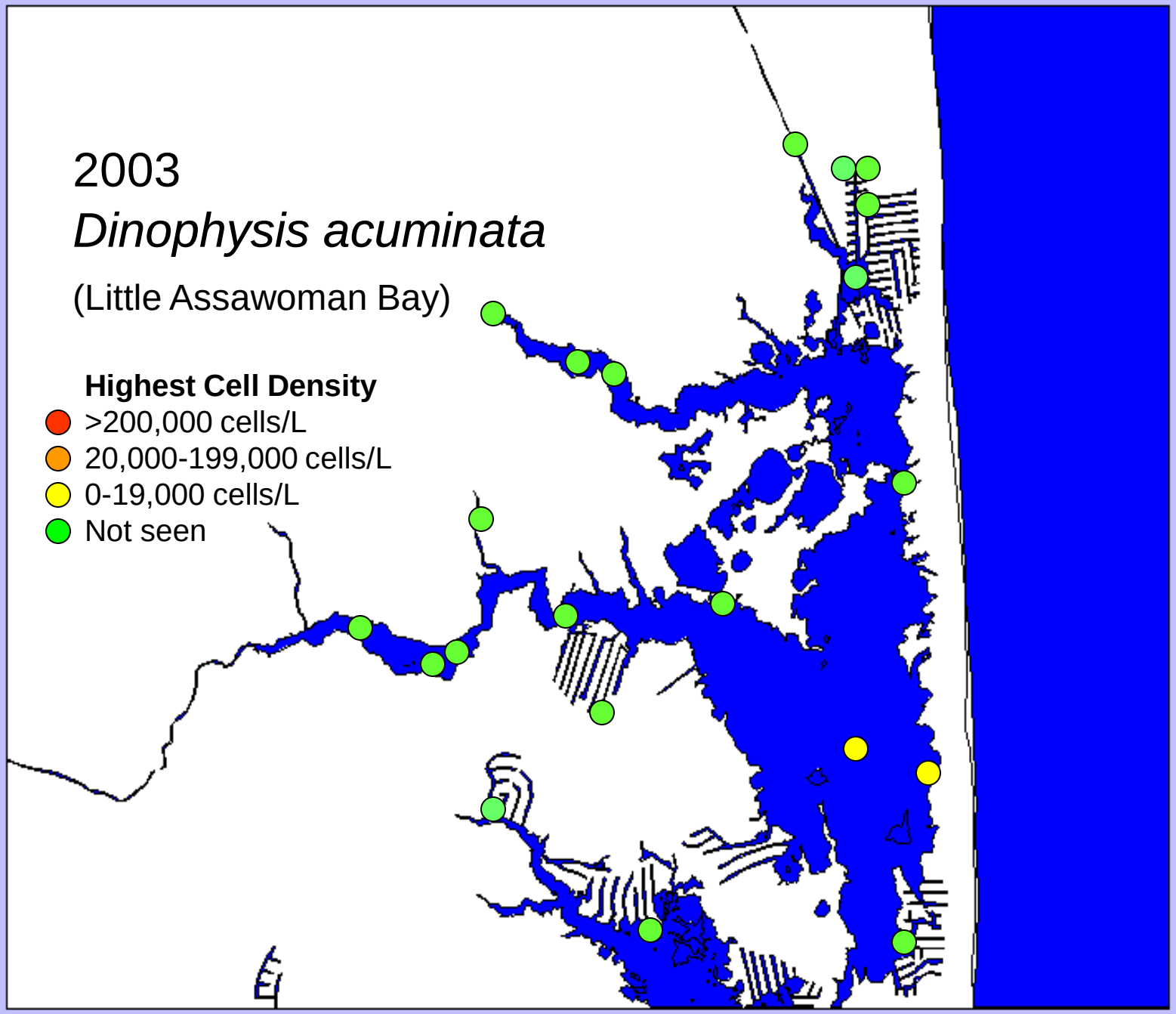

2003
Dinophysis acuminata
(Rehoboth and Indian River Bays)
Highest Cell Density
>200,000 cells/L
20,000-199,000 cells/L
0-19,000 cells/L
Not seen

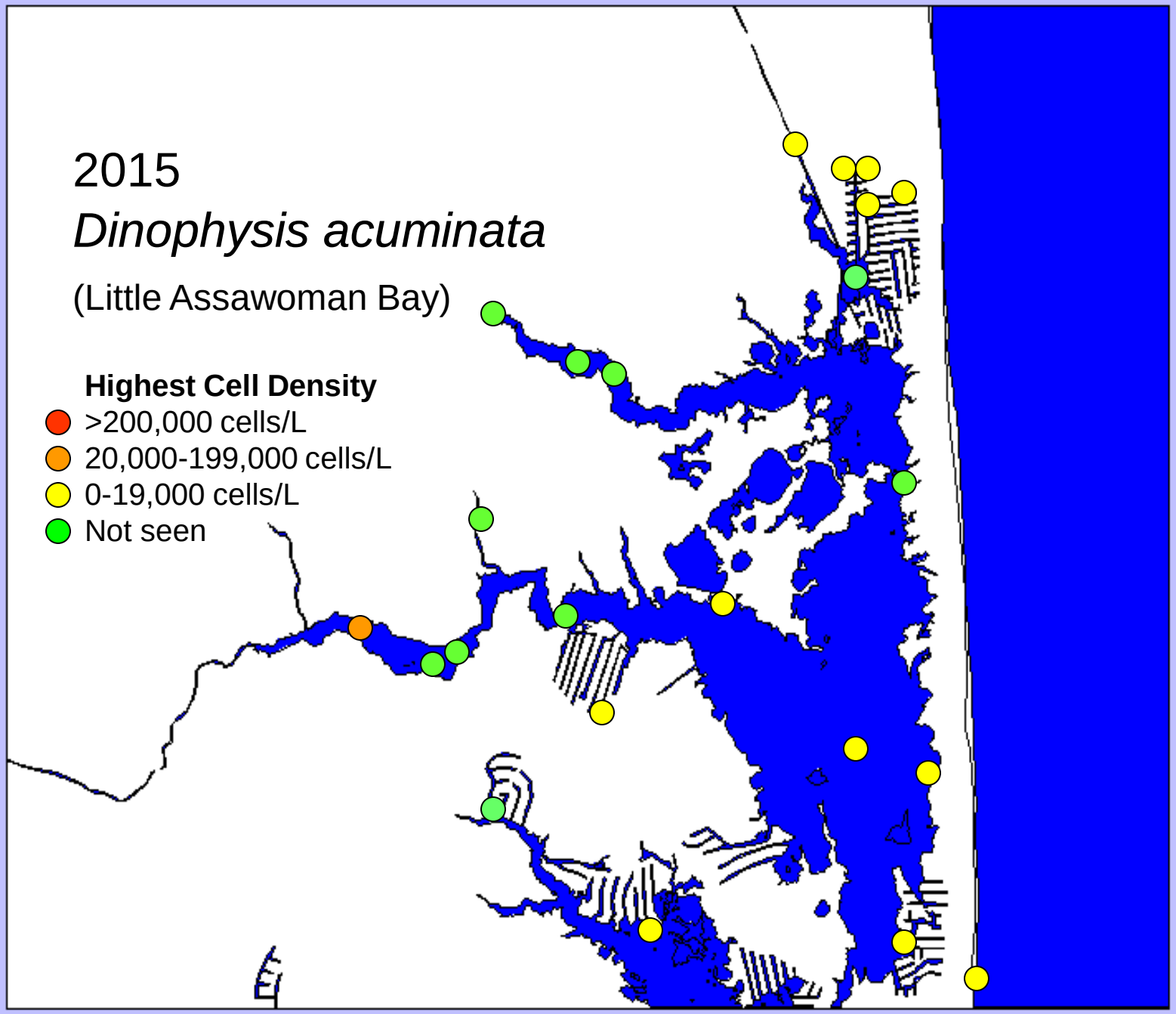
In 2002, MD closed portions of shellfish harvesting areas in the lower Potomac River due to concern about levels of *Dinophysis*, but toxin levels in shellfish were below US FDA regulatory guidelines.

In 2003, a bloom was observed within an approved shellfish area near the mouth of Love Creek in Rehoboth Bay. Concentrated water samples collected by plankton nets were sent to Sherwood Hall (US FDA) for toxin tests. Fortunately, DSP toxins (OA and DTX-1) were not detected.

Methods of toxin extraction and detection have improved since that time.


2015
Dinophysis acuminata
(Rehoboth and Indian River Bays)
Highest Cell Density
>200,000 cells/L
20,000-199,000 cells/L
0-19,000 cells/L
Not seen


2003
Dinophysis acuminata
(Little Assawoman Bay)
Highest Cell Density
>200,000 cells/L
20,000-199,000 cells/L
0-19,000 cells/L
Not seen


2015
Dinophysis acuminata
(Little Assawoman Bay)
Highest Cell Density
>200,000 cells/L
20,000-199,000 cells/L
0-19,000 cells/L
Not seen

By 2013, MD DNR had become concerned about *Dinophysis* levels in the Coastal bays and initiated a sentinel shellfish study in a few locations. Only low levels of 2 forms of toxin were detected in shellfish.

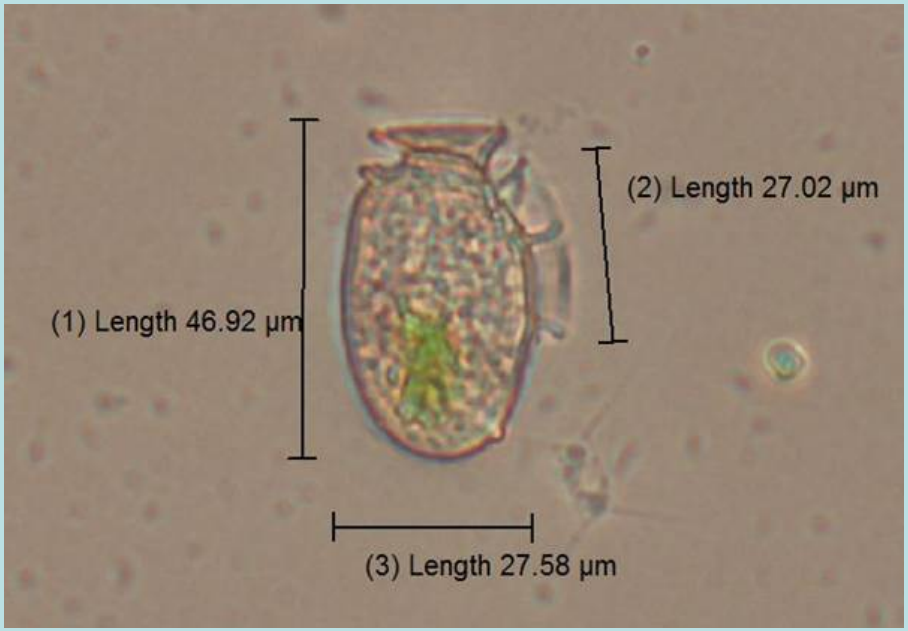
Mike Bott, DE DNREC, participated in the MD sentinel shellfish study with one site in White Oak Creek (Northern Rehoboth Bay). While *D. acuminata* was present intermittently between March and May, cell densities did not exceed 10,000/L, and toxin was barley detectable or non-detectable in shellfish meat.

In Torquay canal, cell densities reached 580,000/L on May 14, and Mike collected oysters from a nearby oyster gardener and toxin levels just topped the guidance level of 16 ug/g of shellfish tissue, but this site is outside of approved shellfish harvesting waters.

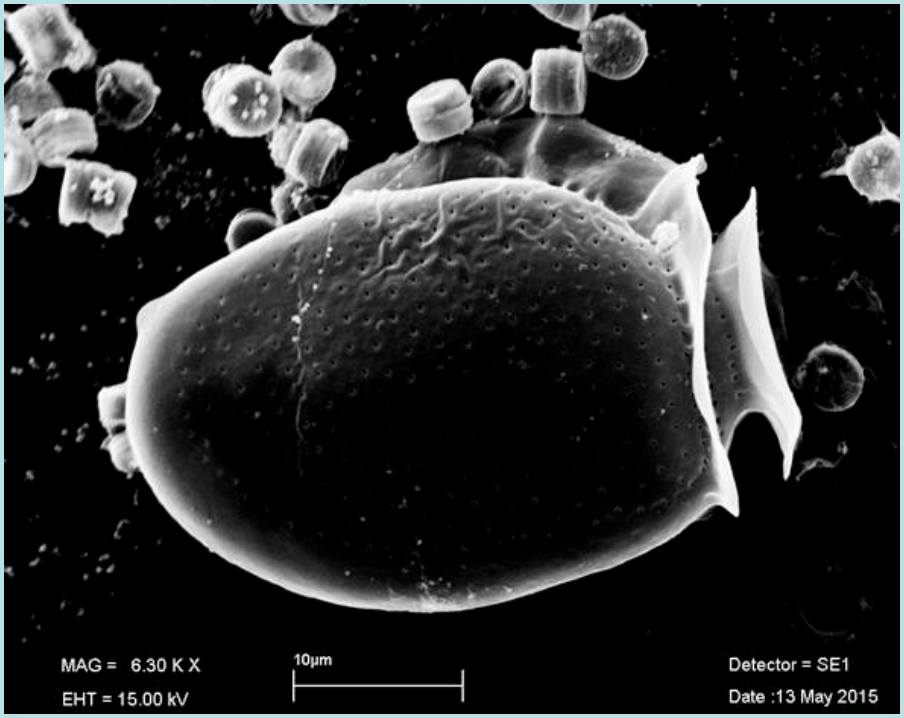
In 2014, a bloom was detected at the mouth of Love Creek with maximum cell density of 87,000/L on May 23. Mike collected clams from the area on May 27, but they showed no toxicity. In Torquay, cell densities reached 640,000/L on May 27.

In 2015, the bloom started in late April in Torquay canal. From Apr 23 to June 1, water samples were filtered for toxin tests. Cell densities reached 645,000/L on May 18, and Mike collected oysters from a nearby oyster gardener on May 26 for toxin tests. We are awaiting results.

More work needs to be done to ensure shellfish safety in the DE Inland Bays, the MD Coastal Bays, the DE Bay, and the Chesapeake Bay.



Light Microscope



Scanning Electron Microscope

Images taken by Todd Egerton